Raman-based nutrient control in bioprocessing

Benefits at a glance

- Real-time glucose control in cell culture bioprocesses achieved using Raman technology
- Raman-based feedback control of glucose optimizes product quality to target product profile
- Higher peak viable cell density and reduced target monoclonal antibody glycation by over 50%
- Raman is a proven Process Analytical Technology (PAT) for Quality by Design (QbD)
- Real-time product and process quality assurance

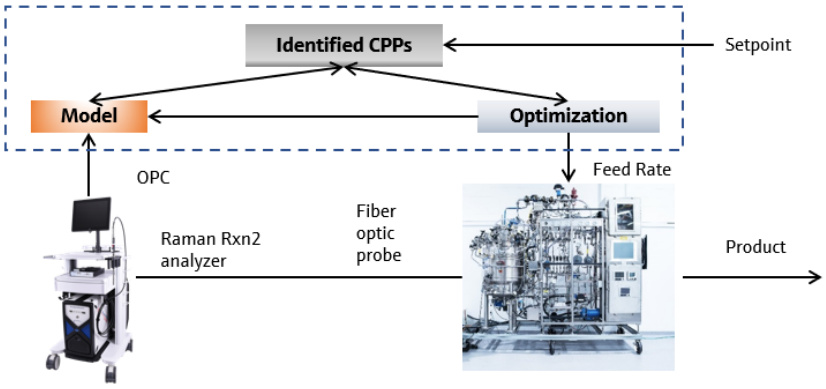

Figure 1: Schematic of Raman-based feedback control

Introduction

The U.S. Food and Drug Administration's Process Analytical Technologies (PAT) and Quality by Design (QbD) initiatives strongly support in-line analytics to ensure process quality before testing of the final product. Production of protein therapeutics by mammalian cells is the most widely used bioprocess because of its ability to properly produce and fold a recombinant protein. 60-70% of biopharmaceuticals are produced by this bioprocess. Bioreactor parameters affect cell metabolic processes and detailed bioreactor knowledge is needed to achieve a balanced and consistent metabolic state of the cells.

Critical process parameters in bioprocessing include pH, dissolved oxygen (DO), temperature, agitation rate, and glucose. Glucose is a critical process parameter because it affects the cell's metabolic profile, production of waste products, and post-translational non-enzymatic glycation of proteins. Glycation can also lead to generation of advanced glycation end products, which results in binding inhibition, loss of therapeutic function, and may generate an unwanted immunogenic response. Glycation can be minimized through careful control of glucose concentration in the process.

Recent research has shown that online measurements of glucose can optimize fed-batch strategies to improve cell density and titer, and increase process robustness. Continuous glucose measurements offer important bioprocess information for process understanding and enables feedback control feed conditions from process development (PD) to manufacturing.

The study by Berry *et al.* describes rapid generation of Raman spectroscopy for feedback control of a CHO cell bioprocess where glycation of the monoclonal antibody (mAb) was identified as a critical quality attribute.[1] This work describes a Raman method to enable automatic glucose setpoint control strategies that minimizes unwanted glycation of the mAb. More specifically, it describes an on-line feedback control system, similar to schematic shown in Figure 1, for glucose concentration based on continuous *in situ* Raman spectral analysis.

ⓘ All Raman analyzers and probes referenced in this application note are Endress+Hauser products powered by Kaiser Raman technology.

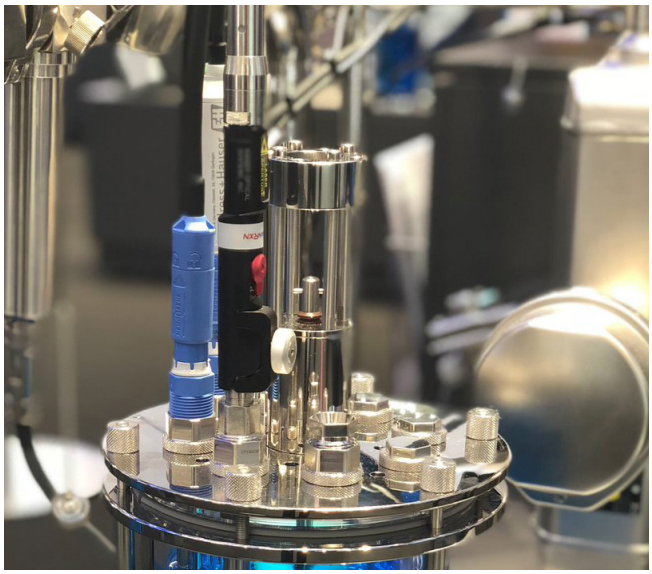

Figure 2: In-line control of product quality achieved from lab to process.

Experimental

As shown in Figure 2, spectra of the process contents were acquired *in situ* using a Raman analyzer equipped with a 785 nm laser, and a Rxn-10 probe with a stainless steel bioprocessing optic. The data were exported automatically to a PLS model in the SIMCA software package. Data collected on-line from the feedback control loop were compared to Raman data collected off-line with a 10X non-contact optic (NCO) and off-line traditional measurements.

Two different strategies of the glucose feedback control system were tested. The first strategy aimed for a constant glucose concentration of 2.25 g/L over the course of the production cycle. The second strategy generated a stepwise decreasing glucose concentration from 9.75 to 2.25 g/L over the same period. In both cases, glucose-free nutrient was provided to the bioreactor daily, and glucose stock solution was added (when needed) in response to on-line Raman data. The glucose concentration in the bioreactor was kept within 0.25 g/L of the setpoint using deadband control. The control logic was initiated when the glucose concentration fell below this value.

Results

A Raman-based glucose control model was used to provide either a constant low glucose concentration to the bioprocess or a stepwise decrease of glucose concentrations with time. The study shows that Raman can integrate into an industrially relevant bioprocess, control bioprocess conditions, and optimize product quality.

Significant glucose control was achieved and quickly integrated into PD work. Since introduction of the Raman-driven control scheme was done early in PD, unintended process changes were identified and corrected quickly, before the process was scaled to manufacturing. One example of a process change was the replacement rate for the complex nutrient feed. This was moved four days in order to avoid precipitation.

Conclusions

Raman spectroscopy is an important PAT solution in cell culture and fermentation bioprocesses. Raman provides *in situ*, simultaneous measurement of multiple critical process parameters with a single probe. The specificity of Raman is a benefit in quick model development and transfer, allowing transfer of the model from lab to manufacturing without significant rework. One example was that Raman measurements indicated changes in the complex nutrient feed over time. With this information, the team was then able to optimize the media replacement schedule in order to avoid precipitation of media components.

References

1. Berry, B. N.; et al. Quick Generation of Raman Spectroscopy Based In-Process Glucose Control to Influence Biopharmaceutical Protein Product Quality during Mammalian Cell Culture. *Biotechnology Progress* **2016**, *32 (1)*, 224–234.